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Influence of row spacing and nitrogen levels on herb and essential oil production and oil quality of *Tagetes minuta* L.

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Abstract

The influence of row spacing and levels of nitrogen application on herb and essential oil production and quality of oil of South American marigold (*Tagetes minuta*) was studied at Lucknow (Uttar Pradesh). Plant rows spaced at 30 cm distance produced 26% and 69% more herb and 20% and 47% more oil than 45 cm and 60 cm spacing, respectively. Nitrogen application led to linear increase in herb and essential oil production. Application of 150 kg N ha^{-1} produced maximum oil (71 kg ha^{-1}) which was 142%, 49% and 23% higher, respectively, over 0, 50 and 100 kg N ha^{-1}. Essential oil biosynthesis was adversely affected in plants that were either spaced closer than 60 cm in rows or received higher quantity of nitrogen. The major chemical component of oil, dihydrotagetone increased with increase in row spacing and sharply decreased with increase in nitrogen level beyond 50 kg ha^{-1}, while a reverse trend was observed for limonene, (Z)-tagetone and (Z)-tagetenone.

Keywords: marigold, nitrogen level, quality, spacing, *Tagetes minuta,* yield.

Marigold (*Tagetes minuta* L.), an important source of essential oil, extensively used in flavour and fragrance industries, is of South American origin but occurs as weed in different parts of the world. Wild collections of *T. minuta* from hills of Himachal Pradesh have been the major source of supply of oil to industries in India. However, the declining population of the plant in the wild state and increased demand of oil necessitated systematic cultivation of *T. minuta* in the country. The Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, developed some of the basic agronomic requirements of *T. minuta* as winter crop for subtropical climate of Uttar Pradesh (CIMAP 2002), and Uttranchal (Ram *et al.* 1998) and semi-arid tropical climate in Karnataka (Rao *et al.* 1999). Under *tarai* region of Uttranchal, transplanting of *T. minuta* at 45 cm x 45 cm yielded better than that at 60 cm x 60 cm and 60 cm x 45 cm spacing (Ram *et al.* 1999), indicating scope for further improvement in yield through increase in plant population densities. Being a foliage rich crop, *T. minuta* may require high level of N fertilization to express the maximum yield potential. Application of 150 kg to 200 kg N ha^{-1} has been suggested to exploit maximum yield potential of *T. minuta* by Ram *et al.* (1999) and Singh (2001). Besides, several factors such as origin of plant (Hethelyi *et al.* 1986), the place of cultivation and nutrition (Graven *et al.* 1991; Singh 2001) are reported to affect the quality of essential oil. Hence, the role of plant row spacing and levels of N application on herb and essential oil production and quality of oil was studied under sub-tropical climate of north Indian plains.

The field experiment was conducted during winter season of 2002–03 on sandy loam soil (pH 7.8) at the research farm of Central Institute of Medicinal and Aromatic Plants, Lucknow (26.5°N, 80.5°E, 120 m altitude) under sub-tropical climate. The soil of the experimental plot had 64.5 kg mineralizable N (alkaline $KMnO_4$ extractable), 14.8 kg available P (0.5 M $NaHCO_3$ extractable), and 53.8 kg ha^{-1} available K (ammonium acetate extractable). The treatments consisting of three row spacings (30 cm, 45 cm and 60 cm) and four N levels (0, 50, 100 and 150 kg ha^{-1}) were tested in a factorial randomized block design with three replications and individual plots measuring 3.6 m x 3.0 m. Forty day old seedlings were planted at 30 cm plant distance in rows on 10 December 2002. Uniform application of 60 kg ha^{-1} each of P_2O_5 and K_2O was done before planting of crop. Nitrogen was applied in three equal splits, at transplanting, 30 and 60 days after transplanting. Harvesting was done at full bloom on 7 April 2003, when *T. minuta* plants contain the maximum essential oil (Singh *et al*. 2006). Fresh biomass yield was recorded. For estimating dry matter content in fresh biomass, a sample of 200 g fresh biomass was taken from each plot at the time of harvest and dried in the oven. Dry biomass yield was calculated by multiplying fresh biomass yield and dry matter content.

Oil content in green herb of *T. minuta* was determined with Clevenger's type apparatus a day before the harvest of experiments. The oil content in dry herb was calculated on moisture free herb basis. The oil yield (kg ha^{-1}) was calculated by multiplying the oil content (%) with dry biomass yield of the respective treatments and the factor 0.90 (approximate specific gravity of oil). To study the effects of treatments on the oil composition, oil samples were subjected to GC analysis on Perkin Elmer GC, model AUTO XL, fitted with PE-5 capillary column (50 m x 0.32 mm) with H_2 inlet pressure of 10 psi and temperature program of 100o C- 220°C @ 3°min. $^{-1}$, initial hold of 1 min. Injector and FID temperatures were kept at 220°C and 250°C, respectively. The data were processed on total Chrom Navigator. The identification of peaks was based on GC-MS analysis on Perkin Elmer Turbo system using the same column and conditions with He at 10 psi as carrier. The Wiley and NIST libraries were used for component identification.

The data recorded on various parameters were statistically analysed using ANOVA and treatment differences were computed at 5% level of probability (Panse & Sukhatme 1967) .

Effect of row spacing

Row spacing had significant influence on dry biomass production, oil biosynthesis, oil yield and major chemical constituents of oil (Table 1). The maximum dry herbage and essential oil yields were associated with 30 cm row spacing. The crop planted at 30 cm row spacing produced 26% and 69% more dry biomass over 45 cm and 60 cm row spacing, respectively; the corresponding increase in essential oil production was 20% and 47%, respectively. A highly negative correlation ($r = -1.0$, $r^2=1.0$) between row spacing and essential oil production was observed. With each centimeter increase in plant row spacing, the reduction in essential oil production was 0.65 kg ha^{-1} ($y=80.75 - 0.65\ x$). The increase in biomass and oil production were probably due to more efficient utilization of natural as well as applied resources at closer row distance. Essential oil content in dry biomass (0.98%) at 60 cm row spacing of *T. minuta* was 9.0% and 10.4% more than 0.90% and 0.87% recorded in 45 cm and 30 cm spacing, respectively (Table 1). These results are in agreement with those reported by Ram *et al.* (1998) in *T. minuta* earlier. At 30 cm and 45 cm row spacing, yellowing of lower leaves due to mutual shading was quite apparent at the time of harvest, and this might have caused loss in essential oil content. Plant row spacing had strong effect on the chemical

Table 1. Dry biomass production, oil content, oil yield and chemical constituents of *Tagetes minuta* as influenced by row spacing and nitrogen levels

Treatment	Dry biomass yield (kg ha^{-1})	Oil content (% v/w) DWB	Oil yield (kg ha^{-1})	Oil constitution (%)					
				Limonene + (Z)-β-ocimene	Dihydrotage-tone	(E)-tagetone	(Z)-tagetone	(E)-tagetenone	(Z)-tagetonone
Row spacing (cm)									
30	8147.5	0.87	61.5	12.3	42.8	3.3	19.2	3.1	12.1
45	6454.2	0.90	51.1	10.9	47.5	3.5	15.5	3.2	10.4
60	4822.5	0.98	41.9	10.3	49.8	3.7	12.5	2.8	5.7
CD (P=0.05)	378.3	0.02	2.85	0.6	4.6	0.4	1.3	0.3	0.6
Nitrogen level (kg ha^{-1})									
0	3373.3	0.98	29.3	6.4	62.3	3.2	8.9	2.7	5.3
50	5742.2	0.94	47.8	7.6	57.0	3.3	12.2	2.9	7.9
100	7431.1	0.90	57.9	12.8	42.6	4.0	18.3	3.1	10.6
150	9352.2	0.85	71.0	17.9	24.7	3.5	23.6	3.4	13.6
CD (P=0.05)	436.8	0.023	3.29	0.7	5.5	0.45	1.45	0.34	0.7

v /w =Volume/weight (ml 100 g^{-1} biomass); DWB=Dry weight basis composition of essential oil. The major constituent of the oil, dihydrotagetone considerably decreased, while limonene, (Z)-tagetone and (Z)-tagetenone increased with decrease in plant row spacing (Table 1).

Effect of nitrogen levels

T. minuta responded favourably to N fertilization (Table 1). Application of 50, 100 and 150 kg N ha^{-1} increased the dry herb production by 70%, 120% and 177% and essential oil by 63%, 98% and 142%, respectively, over control. The essential oil production increased linearly ($y=31.25 + 0.27 x$) and each kg increase in N dose led to 0.27 kg ha^{-1} increase in essential oil production. Essential oil content in dry biomass decreased significantly with increase in N levels. The response of *T. minuta* to high level of N application is understandable in view of its very fast growth and foliage rich plant canopy. Response up to 150 kg N ha^{-1} in *tarai* soils of Uttaranchal (Ram *et al*. 1999) and up to 200 kg N ha^{-1} in red sandy loam soils in semi-arid tropics of Bangalore has been reported (Singh 2001). The major constituents of essential oil, dihydrotagetone declined sharply with increase in N level beyond 50 kg ha^{-1}, while limonene, (Z)-tagetone and (Z)-tagetenone increased (Table 1). Similar results have been reported by Graven *et al*. (1991).

The results of the study suggest that in sub-tropical climate, *T. minuta* should be planted at 30 cm row spacing and fertilized with 150 kg N ha^{-1} or more to obtain higher oil yield. The study offers the opportunity to harvest essential oil rich in desired aroma chemicals through manipulation in agronomic practices.

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